Purification and Characterization of Arcelin Seed Protein from Common Bean[1]

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ABSTRACT

Arcelin, a seed protein originally discovered in wild bean accessions, was purified, characterized, and compared to phaseolin, the major seed protein of common bean, and to phytohemagglutinin (PHA), the major bean seed lectin. Arcelin and PHA has several characteristics in common. Both were glycoproteins having similar subunit M_r, deglycosylated M_r, and amino acid compositions. The two proteins were related antigenically and they had the same developmental timing of accumulation. Arcelin also had some hemagglutinating activity, a characteristic associated with lectins. However, several features distinguished arcelin from PHA. Arcelin had a more basic isoelectric point than PHA, greater numbers of basic amino acid residues, additional cysteine residues, and one methionine residue, which PHA lacks. Native PHA protein is a tetramer of subunits, and although a small component of native arcelin protein was also tetrameric, most of the arcelin preparation was dimeric. The hemagglutinating activity of arcelin was specific only for some pronase-treated erythrocytes. It did not agglutinate native erythrocytes, nor did it bind to thyroglobulin or fetuin affinity resins as did PHA. Although arcelin has lectin-like properties, we believe the distinctions between arcelin and PHA warrant the designation of arcelin as a unique bean seed protein.

The major seed storage proteins of cultivated common bean (*Phaseolus vulgaris*) are phaseolin and PHA,[2] also referred to as bean lectin. In several wild bean accessions, a third important protein fraction has been observed which had not been described previously in bean cultivars (19, 23). This fraction was discovered as a major seed protein having a different electrophoretic mobility than either phaseolin or PHA with which it occurred in seeds of some wild beans. Four electrophoretic variants of this protein were observed which consisted of distinct groups of polypeptides having similar mobilities on two-dimensional gels. This protein was named arcelin and the variants were designated arcelin-1, 2, 3, and 4.

There has been only limited characterization of arcelin, but the protein and its genetic control have several interesting features. Arcelin was shown to occur in the globulin-2 protein fraction and the presence of arcelin in bean seed was correlated with greatly reduced phaseolin content (23). Arcelin was found to be controlled genetically in a simple Mendelian fashion. The expression of alleles for the presence of different arcelin variants

was co-dominant with respect to each other and dominant with respect to alleles for the absence of arcelin. Genes controlling arcelin expression were tightly linked (<0.3% recombination) to those controlling PHA expression. The presence of arcelin in wild bean accessions was correlated with high levels of resistance to two bruchid beetle species (19, 24).

Although arcelin protein has a different electrophoretic mobility than other major seed proteins, it is not known whether arcelins represent a truly distinct class of seed proteins. The relationship of arcelin to other seed proteins is particularly interesting in light of the tight linkage between arcelin and PHA genes. In this study, we report on the purification and characterization of the arcelin-1 variant and compare its properties to those of PHA and phaseolin seed proteins.
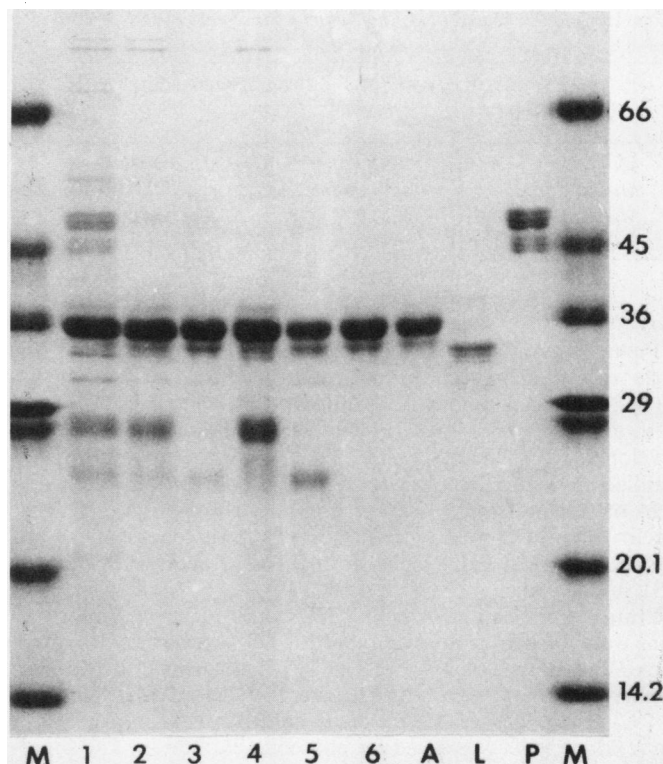

FIG. 1. Crude, partially purified, and purified seed proteins of SARC1-7 separated by SDS-PAGE. Samples shown are crude seed proteins extracted with 0.5 M NaCl (lane 1) or 10 mM NaCl, pH 2.4 (lane 2), partially purified proteins globulin-a (lane 3), albumin-a (lane 4), globulin-b (lane 5), or albumin-b (lane 6) and purified proteins arcelin (lane A), PHA (lane L), or phaseolin (lane P). Lanes M contain mol wt marker proteins (mol wt indicated on the right in kD).

[2] Abbreviations: PHA, phytohemagglutinin; PBS, phosphate-buffered saline (0.15 M NaCl, 3.6 mM sodium phosphate, pH 7.4); ddH$_2$O, deionized distilled water.
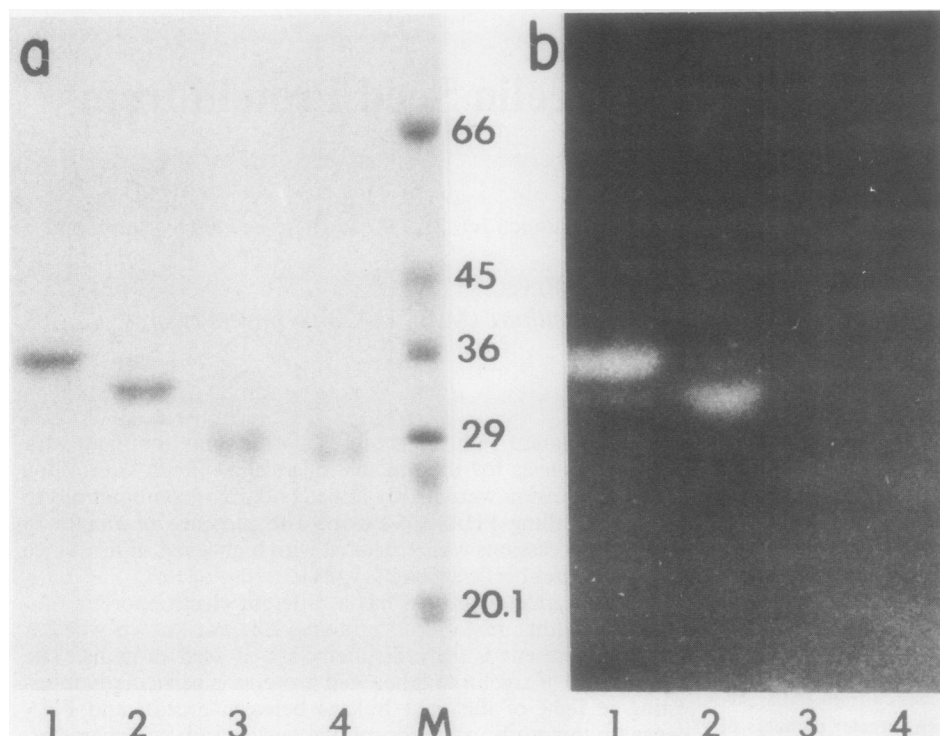
FIG. 2. Separation of arcelin (lane 1), PHA (lane 2), deglycosylated arcelin (lane 3), and deglycosylated PHA (lane 4) by SDS-PAGE. Panel a shows a gel stained for total protein and panel b is a gel stained for carbohydrate.

Table I. *Relative Mol Wt (M_r) and Isoelectric Points of Purified Arcelin and PHA*

	Bean Seed Protein	
	Arcelin	PHA
M_r native	80,900; 159,600	146,100
M_r denatured	35,800–37,400	32,700–40,700
M_r deglycosylated	29,900	29,000; 30,800
Isoelectric point (pH)	6.7; 6.8	5.2–5.4

MATERIALS AND METHODS

Plant Materials. Three near-isogenic lines of *Phaseolus vulgaris* with different seed protein compositions were used for this study. 'Sanilac' is a navy bean cultivar that contains both phaseolin and PHA seed proteins, but no arcelin. L12-56 is a line developed by backcrossing the allele for PHA deficiency into 'Sanilac' (16), and therefore contains phaseolin but no detectable PHA or arcelin. Line, SARC1-7, containing phaseolin, PHA and arcelin, was developed by backcrossing the arcelin-1 allele and the tightly linked PHA locus from the wild bean line, UW325 (23), into 'Sanilac.'

Protein Purification. Arcelin, PHA, and phaseolin seed proteins were purified from seed of SARC1-7. Bean flour (10 g) was stirred in 100 ml of 10 mM NaCl (pH 2.4) for 1 h at room temperature, then the mixture was centrifuged (30,000g, 20 min, at 4°C). The pellet was reextracted and centrifuged as above and the residual pellet was saved for phaseolin extraction. The supernatants were combined (180 ml) and dialyzed for 24 h at 4°C against three changes of 6 L ddH₂O. The precipitate (globulin-a) was pelleted by centrifugation and the globulin-a and supernatant (albumin-a) were lyophilized. Globulin-a (50 mg) was dissolved in 10 mM NaCl (pH 2.4), centrifuged and the supernatant was dialyzed against ddH₂O as before. The protein that precipitated after dialysis (globulin-b) was pelleted by centrifugation and the globulin-b and supernatant (albumin-b) were lyophilized. PHA

was removed from the albumin-b fraction by Sepharose-thyroglobulin or Sepharose-fetuin affinity chromatography (17). The protein that did not bind to the affinity ligand was shown electrophoretically to be highly purified arcelin. PHA bound to the affinity ligand and after extensive washing with PBS, it was eluted with 0.5 M NaCl containing 0.1 M glycine (pH 3.0). The arcelin and PHA were dialyzed against ddH₂O and lyophilized.

Phaseolin was extracted from the residual pellet by stirring in 0.5 M NaCl (pH 2.4) for 30 min at room temperature. This mixture was centrifuged and the supernatant was dialyzed at 4°C against ddH₂O. The precipitant was pelleted by centrifugation, washed with cold ddH₂O, redissolved in 0.5 M NaCl, centrifuged, and the supernatant was dialyzed at 4°C against ddH₂O. This was repeated twice and the final pellet (phaseolin) was lyophilized. Protein samples were dissolved in PBS, except phaseolin which was dissolved in 0.5 M NaCl, for electrophoresis and hemagglutination assays.

Electrophoresis. Protein samples were mixed with an equal volume of cracking buffer (0.625 M Tris-HCl [pH 6.8], 2 mM EDTA, 2% [w/v] SDS, 40% [w/v] sucrose, 1% [v/v] β-mercaptoethanol, 0.01% [w/v] bromophenol blue) and placed in a boiling water bath for 5 min. Proteins were separated by SDS-PAGE in 15% polyacrylamide slab gels (11, 12). Gels were stained with Coomassie brilliant blue R-250 (1.5% [w/v] in 45% [v/v] ethanol, and 9% [v/v] acetic acid) and destained with a solution of 20% (v/v) ethanol, 6% (v/v) acetic acid. The M_r values of denatured and deglycosylated arcelin and PHA were calculated from comparisons to the electrophoretic mobilities of standard proteins. The isoelectric point of purified arcelin was determined by isoelectric focusing in polyacrylamide tube gels containing 8 M urea (15). The ampholines used, pH 5 to 8 (LKB) and pH 3 to 10 (BioRad), generated a pH gradient of 4.5 to 7.8 as determined by measuring the pH of 0.5 cm gel slices after soaking in 1 M KCl for 3 h.

Gel filtration. The M_r of native arcelin and PHA proteins were determined by filtration through a Sephacryl S-300 (Pharmacia)
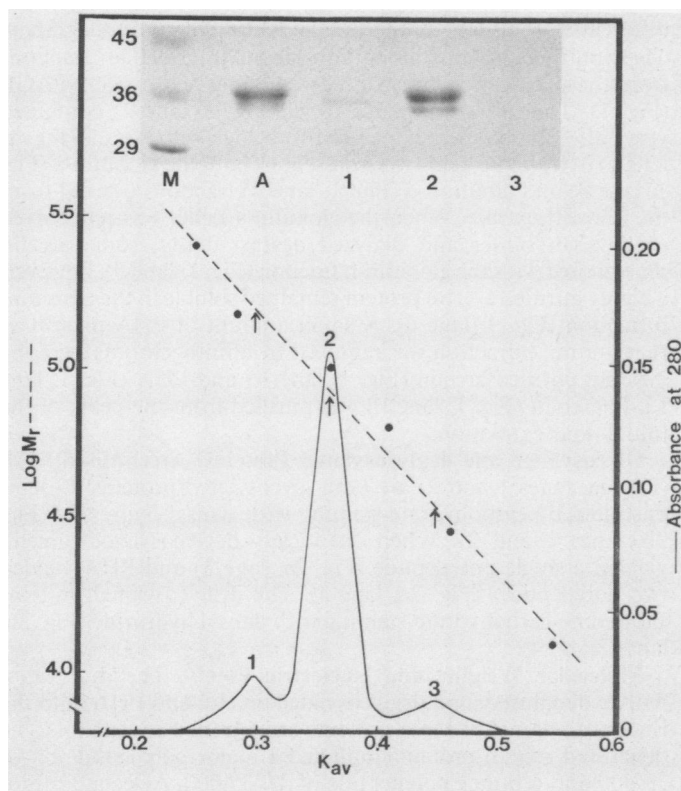
FIG. 3. Gel filtration of purified arcelin on Sephacryl S-300. Mol wt markers were catalase (250,000), alcohol dehydrogenase (150,000), uricase (100,000), hemoglobin (64,500), carbonic anhydrase (29,000), and Cyt c (12,400). Arrows indicate M_r of native arcelin proteins. Proteins from the eluted peaks were separated by SDS-PAGE: purified arcelin (lane A), peak 1—tetramer (lane 1), peak 2—dimer (lane 2), and peak 3 (lane 3).

column (1.5 × 118 cm) with a flow rate of 6.45 ml/h and monitoring the absorbance of the effluent at 280 nm. PBS was used as the equilibration, elution, and sample dissolving buffer.

Deglycosylation and Detection of Glycoproteins. Purified arcelin and PHA were chemically deglycosylated using trifluoromethane sulfonic acid (4). Arcelin was treated in the acid solution for 1 h at room temperature and PHA was treated for 3 h at 4°C. Deglycosylated samples were lyophilized and dissolved in cracking buffer for SDS-PAGE. After SDS-PAGE, gels were fixed with a solution of 5% (v/v) formaldehyde and 25% (v/v) ethanol. Gels were rinsed twice in 50% (v/v) methanol and twice in ddH$_2$O and then successively treated with paraperiodic acid, sodium metabisulfite, acidic dimethylsulfoxide, and dansyl hydrazine (3). Carbohydrates were visualized and photographed under UV light. A control gel, omitting the paraperiodate oxidation, was included and showed no detectable staining.

Amino Acid and Sugar Analysis. Purified arcelin was analyzed for amino acid composition by Allan Smith, University of California, Davis. All amino acids, except cysteine and tryptophan, were separated using a Durrum D-500 amino acid analyzer after a 24 h hydrolysis in 6 N HCl at 110°C under nitrogen. Cysteine was determined as cysteic acid after performate oxidation. Tryptophan was quantified by the acid-ninhydrin method (6), and compared to the protein content as estimated by the biuret method (7). Glucosamine and galactosamine were estimated by extrapolating to time zero the values obtained on a Beckman 6300 amino acid analyzer after 4 and 6 h hydrolyses in 4 N HCl at 100°C. Neutral sugars were estimated by the phenol-sulfuric acid method (2) using mannose as standard.

Table II. *Chemical Composition of Major Bean Seed Storage Proteins*

Amino Acid	Amino Acids		
	Arcelin	PHA[a]	Phaseolin[b]
	mol %		
Asx	14.9	12.5	11.9
Thr	7.3	9.6	4.2
Ser	11.5	14.4	9.4
Glx	8.2	6.7	15.2
Pro	3.3	3.1	3.7
Gly	5.7	6.8	5.2
Ala	6.1	6.6	4.9
Cys	1.1	0.2	0.3
Val	7.1	7.7	6.5
Met	0.2	0.0	0.7
Ile	4.7	5.4	6.3
Leu	6.7	8.9	10.2
Tyr	3.0	2.3	2.8
Phe	6.5	5.7	5.9
Lys	5.9	4.7	5.6
His	2.4	1.1	2.5
Arg	4.7	2.4	4.3
Trp	0.9	2.0	0.6
	g/100g glycoprotein		
Neutral sugars	16.2	9.1	4.5
Glucosamine	3.7	1.4[c]	1.0
Galactosamine	0.0		

[a] Previously published data (14). [b] Derived from previously published data (21). [c] Total amino sugars.

Cyanogen Bromide Cleavage. Purified arcelin was cleaved into peptides using cyanogen bromide as described previously (8), except that 72% (v/v) formic acid was used instead of 0.1 N HCl. The reaction was stopped after 20 h by freezing at −80°C, followed by lyophilization. Digestion products were separated by SDS-PAGE using Tris-borate as running buffer (5). After electrophoresis, the gel was formaldehyde-fixed and stained with Coomassie brilliant blue R-250 (25).

Hemagglutination Assays. The hemagglutinating activities of purified proteins and crude bean extracts were determined using erythrocytes from rabbit, mouse, human type A, guinea pig, rat, and cow blood. Erythrocytes were prepared as described previously (1) using the same procedure to treat cells with pronase as was described for the trypsin treatment of cells. Crude protein extracts were obtained by stirring bean flour in PBS (2% w/v) for 1 h at room temperature followed by centrifugation (12,000g, 4°C, 20 min). Crude extracts and purified proteins (2 mg/ml) were serially diluted with equal volumes of PBS and 50 μl of each dilution step was mixed with 50 μl of native or treated erythrocytes (3% v/v PBS) in wells of microtiter plates. Hemagglutinating activity was scored visually after 1 h at room temperature.

Immunoblotting. Rabbit antibodies were raised against purified arcelin by injecting subcutaneously 100 μg of arcelin with Freund's complete adjuvant, followed 4 weeks later with a second injection containing 100 μg arcelin and Freund's incomplete adjuvant and a third injection 1 week later. The rabbit was bled 3 d after the last injection and the serum was frozen. Mouse serum containing antibodies to deglycosylated PHA was kindly provided by Leslie Hoffman, Agrigenetics Corporation, Madison, WI. Immunoblotting of proteins separated by SDS-PAGE was performed as described previously (26), except that the blocking solution was 1% (w/v) BSA, 5% (w/v) nonfat dried milk, 20 mM Tris-HCl (pH 7.5), and 0.15 M NaCl. Primary antiserum was used in a $^1/_{500}$ dilution in the blocking solution and secondary antibody was either goat anti-rabbit or rabbit anti-mouse immunoglobulin G conjugated to alkaline phosphatase (Sigma) (1
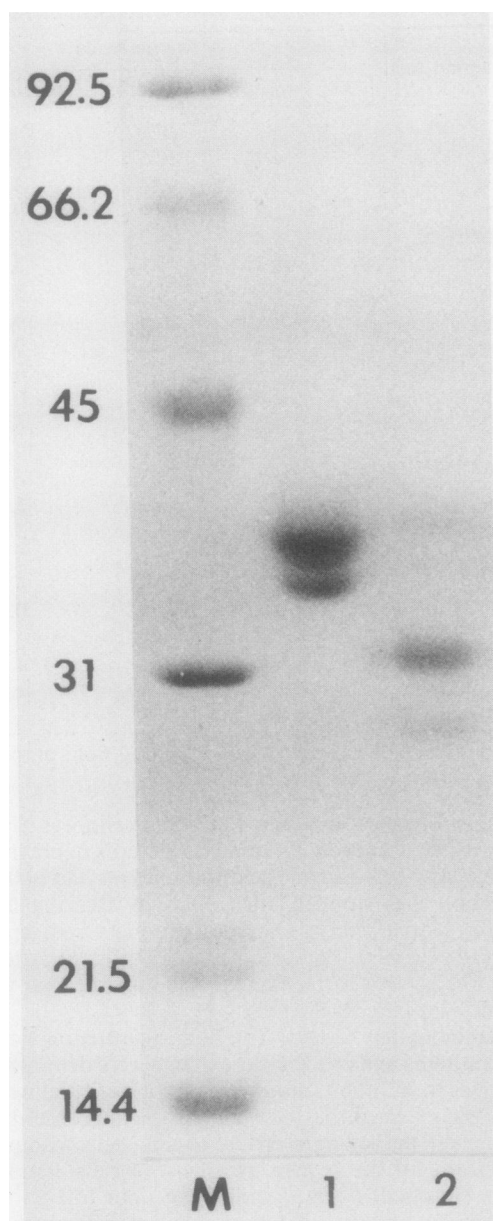
FIG. 4. Separation of arcelin (lane 1) and cyanogen bromide cleaved arcelin (lane 2) by SDS-PAGE.

μl/ml blocking solution). Proteins were visualized colorimetrically using the phosphatase substrates nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (26). A control immunoblot treated with rabbit preimmune sera showed no detectable reaction.

Developmental Study. Developing seeds of 'Sanilac,' L12-56 and SARC1-7 bean lines were harvested from greenhouse plants grown under natural light beginning 10 d after flowering up until pod maturity (36 d after flowering). Seeds were frozen in liquid N_2 and stored at $-80°C$. Cotyledons from each sample were ground in 0.5 M NaCl, 50 mM sodium phosphate (pH 7.2) (10% w/v) using a mortar and pestle. One ml of mixture was centrifuged and the supernatant was stored at $-80°C$. The samples were diluted with an equal volume of 0.5 M NaCl (three volumes for mature seeds) and separated by SDS-PAGE.

RESULTS

Protein Purification. Three major protein fractions, arcelin, PHA, and phaseolin, were purified from seed of SARC1-7 using differential solubility properties and affinity chromatography. These purified proteins, along with various intermediate fractions from the purification procedure, were separated by SDS-PAGE (Fig. 1). The initial low salt extract (Fig. 1, lane 2) contained very little phaseolin as compared to a high salt extract (Fig. 1, lane 1). Arcelin proteins were present in both the globulin-a (Fig. 1, lane 3) and albumin-a (Fig. 1, lane 4) fractions derived from the low salt extract. When the globulin-a pellet was redissolved in low salt buffer and dialyzed against dH_2O, some arcelin precipitated with the globulin-b fraction (Fig. 1, lane 5); however, a highly purified arcelin protein remained soluble in the albumin-b fraction (Fig. 1, lane 6). A small amount of PHA present in the albumin-b fraction was removed by affinity chromatography yielding purified arcelin (Fig. 1, lane A) and PHA (Fig. 1, lane L). Phaseolin (Fig. 1, lane P) was purified from the pellet of the initial flour extraction.

Glycosylated and deglycosylated Proteins. Arcelin and PHA (Fig. 2a, lanes 1 and 2) are both glycosylated proteins as demonstrated by carbohydrate-staining with dansyl hydrazine (Fig. 2b, lanes 1 and 2). When chemically deglycosylated, arcelin yielded a single polypeptide (Fig. 2a, lane 3) and PHA yielded two polypeptides (Fig. 2a, lane 4). The deglycosylated proteins showed no carbohydrate-staining with dansyl hydrazine (Fig. 2b, lanes 3 and 4).

Molecular Weights and Isoelectric Points. The mol wt of native, denatured, and deglycosylated arcelin and PHA, and the isoelectric points of these proteins are reported in Table I. The denatured arcelin protein contained a major polypeptide of M_r 37,400 and a minor polypeptide of M_r 35,000. A second minor polypeptide having an intermediate M_r sometimes could be resolved by SDS-PAGE (Fig. 3). Two-dimensional isoelectric focusing/SDS-PAGE of purified arcelin also showed one major and two minor polypeptides, all of which had similar electrophoretic mobilities (gel not shown). The denatured PHA protein contained a major polypeptide of M_r 34,300 and several minor polypeptides with M_rs ranging from 32,700 to 40,700. The deglycosylated forms of arcelin and PHA had very similar M_r values ranging from 29,000 to 30,800. The isoelectric points of arcelin and PHA were significantly different; arcelin focused at two pH values, 6.7 and 6.8; whereas PHA focused in the range of pH 5.2 to 5.4.

The mol wt of native proteins were determined by gel filtration. PHA eluted as a single peak with a M_r of 146,000, corresponding to a tetramer of polypeptide subunits. The native arcelin protein eluted as a minor peak with M_r of 159,600 and a major peak with M_r of 80,900, corresponding to the tetramer and dimer of polypeptide subunits, respectively (Fig. 3). Polypeptide components of these two native forms had different electrophoretic mobilities. The tetrameric peak consisted of the higher M_r minor polypeptide (Fig. 3, lane 1), and the dimeric peak consisted of the major 37,400 M_r subunit and the lower M_r minor subunit (Fig. 3, lane 2). A third, minor peak contained no protein visible by Coomassie blue staining (Fig. 3, lane 3).

Chemical Compositions. The results of amino acid and sugar analyses of arcelin together with the published analyses of PHA and phaseolin are shown in Table II. The amino acid compositions of all three proteins had some similarities; however, those of arcelin and PHA were more similar to each other than to the composition of phaseolin. Arcelin differed from PHA in that it contained some methionine (<1 residue/subunit), more cysteine and more basic amino acid residues than did PHA. Arcelin also contained more sugar residues than either PHA or phaseolin, and based on the percentage neutral sugars and glucosamine, the predicted deglycosylated mol wt of arcelin (29,900) agreed exactly with the M_r of the deglycosylated arcelin (Fig. 2; Table I).

The presence of at least one methionine residue in the arcelin protein also was detected by cyanogen bromide cleavage (Fig. 4).

Table III. *Agglutinating Activity of Crude Bean Seed Extracts and Purified Proteins with Erythrocytes from Different Animal Sources*

Sample	Rabbit		Mouse		Human		Guinea pig		Rat		Cow		
	N[a]	P[b]	N	P	N	P	N	P	N	P	N	P	T[c]
Crude extracts													
L12-56	0[d]	0	0	0	0	0	0	2	0	ND	0	2	1
Sanilac	8	10	7	12	8	12	9	10	6	≥3	0	5	6
SARC1-7	7	10	6	12	6	13	7	8	5	ND	0	8	9
Purified protein													
PHA	9	10	9	12	8	12	9	10	7	>3	0	10	11
Albumin-b	3	7	ND	8	2	10	4	ND	ND	ND	0	ND	8
Arcelin	0	5	0	7	0	8	0	0	0	1	0	1	2
Phaseolin	0	0	0	0	0	0	0	0	0	ND	0	0	0
Arcelin, 2nd pass	0	5	ND	ND	0	7	0	ND	ND	ND	0	ND	2

[a] N, native erythrocytes. [b] P, pronase-treated erythrocytes. [c] T, trypsin-treated erythrocytes. [d] Numbers indicate last dilution step with hemagglutinating activity (higher number indicates more activity; 0, no hemagglutinating activity observed; ND, not determined).
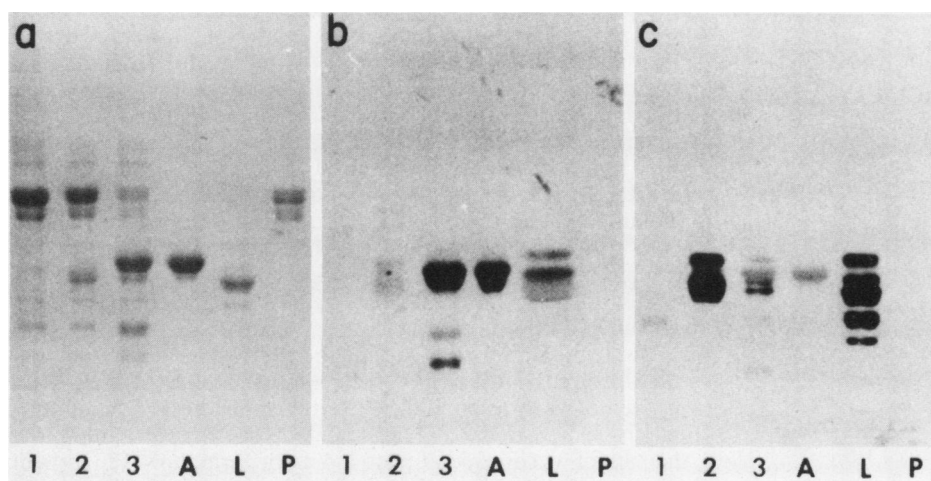

FIG. 5. Crude and purified seed proteins separated by SDS-PAGE and stained with Coomassie blue (panel a) or immunoblotted and reacted with antisera made against purified arcelin (panel b) or deglycosylated PHA (panel c). Samples shown are L12-56 crude extract (lane 1), 'Sanilac' crude extract (lane 2), SARC1-7 crude extract (lane 3), arcelin (lane A), PHA (lane L), and phaseolin (lane P).

The primary cleavage product had a M_r of 32,000. A minor cleavage product ($M_r = 29,000$) also was observed which probably corresponded to a cleavage product from the minor arcelin polypeptide ($M_r = 35,800$). If arcelin protein has a single methionine residue, cleavage products of approximately M_r of 5,000 would also be expected. A peptide this small would have migrated at the front of the gel.

Hemagglutination Assays. The hemagglutinating activities of crude and purified proteins are shown in Table III. Erythrocytes treated with pronase or trypsin were more sensitive to agglutination as has been reported for purified PHA (20) and crude bean extracts (10). Crude extracts of L12-56, which lacks arcelin and PHA, had very low agglutinating activities with only two cell types treated with pronase or trypsin. The crude extract of 'Sanilac,' which contains PHA, had high levels of activity for all native and treated erythrocytes tested, except for native cow cells. This was also observed for the SARC1-7 extract, which contained arcelin and small quantities of PHA. PHA protein purified from SARC1-7 had strong agglutinating activity with both native and pronase- or trypsin-treated cells and activity also was observed in the albumin-b fraction, which contained mostly arcelin and a small amount of PHA. Arcelin protein (purified from albumin-b by removing PHA) did not agglutinate any of the native erythrocytes, but had strong agglutinating activity with pronase-treated rabbit, mouse, and human cells and weak activity with

pronase-treated rat and cow cells and trypsin-treated cow cells. No hemagglutinating activity was observed for purified phaseolin.

The observed hemagglutinating activity of purified arcelin could have been due to a small amount of PHA contamination. To test this possibility, purified arcelin was passed a second time through the Sepharose-thyroglobulin affinity resin and retested for hemagglutinating activity (arcelin, 2nd pass). There was no significant reduction in agglutinating activity for the cells tested, indicating that the observed activity was not due to PHA contamination. The ability of arcelin to agglutinate some treated cells also can be inferred by comparing the SARC1-7 crude extracts and purified PHA for their relative activity with native and pronase-treated cells. Some component of the SARC1-7 crude extract (*i.e.* arcelin) is responsible for a greater activity with pronase-treated *versus* native rabbit, mouse, and human cells than was observed for purified PHA. The relative differences between native and pronase-treated guinea pig cells, which did not react with arcelin, were the same for SARC1-7 crude extract and PHA.

Immunoblots. Immunoblot analyses of crude and purified proteins using antibodies against purified arcelin are shown in Figure 5b. These antibodies reacted strongly with purified arcelin (Fig. 5b, lane A) and with arcelin in the crude extract (Fig. 5b, lane 3). Two lower mol wt proteins in the crude extract also

proteins in the SARC1-7 extract. These antibodies weakly bound a protein in the L12-56 extract which also appeared to be present in the SARC1-7 extract. This protein may represent the low-abundance lectin isolated from PHA-deficient 'Pinto UI 111' seeds (22).

Developmental Accumulation. The accumulation of arcelin, PHA and phaseolin proteins was studied using developing seeds of the three near-isogenic backcross lines: L12-56 (containing phaseolin). 'Sanilac' (containing phaseolin and PHA) and SARC1-7 (containing phaseolin, PHA and arcelin). Proteins extracted from seeds at each developmental stage were separated by SDS-PAGE (Fig. 6). In the L12-56 line, most of the phaseolin accumulated between 12 and 24 d after flowering. A similar accumulation profile was observed for both phaseolin and PHA proteins in 'Sanilac.' In the SARC1-7 line, phaseolin accumulated between 12 and 20 d after flowering, whereas most of the arcelin protein accumulated between 12 and 24 d after flowering.

DISCUSSION

The purpose of this study was to purify and characterize arcelin and compare its properties to those of PHA and phaseolin seed proteins in order to determine if arcelin represents a new type of seed protein. Results of the purification procedure demonstrated that arcelin behaved both as an albumin and globulin seed protein. In fact, arcelin that initially occurred in the globulin-a fraction was partitioned later into albumin-b and globulin-b fractions. These results contrast with those of Romero *et al.* (23) who reported that arcelin behaved as a globulin protein. However, PHA also has been shown to occur in both albumin and globulin protein fractions (18) and in this study, it co-purified with arcelin in the albumin-b fraction. Arcelin, unlike PHA, did not bind to Sepharose-thyroglobulin or Sepharose-fetuin affinity resins; therefore, these affinity ligands were used to separate arcelin and PHA proteins.

The subunit mol wt of arcelin and PHA were very similar and their deglycosylated mol wt were almost identical. Native PHA had a mol wt corresponding to a tetramer of polypeptide subunits as reported previously (13, 17). Most of the native arcelin protein had a mol wt corresponding to a dimeric form; however, a small amount of a tetrameric form also was observed. The subunit components were different for these native conformations suggesting that the three arcelin subunits which had similar mol wt and isoelectric points were not randomly associated with each other in the native protein. One property distinguishing arcelin from PHA was arcelin's more basic isoelectric point which had been reported previously (19). Although the amino acid compositions were very similar, arcelin had more of the basic amino acids (lysine, histidine, and arginine) than did PHA which could account for arcelin's more basic isoelectric point.

The mature PHA protein contains no methionine residues as determined from the nucleotide sequence of genomic DNA clones (9). The results of cyanogen bromide cleavage of purified arcelin indicated that arcelin contained at least one methionine residue. The amino acid analysis of arcelin also showed the presence of a small amount of methionine.

One of the distinct characteristics of PHA is its ability to agglutinate mammalian erythrocytes. Purified arcelin was tested for this property using native, pronase- or trypsin-treated erythrocytes from various animal sources. No hemagglutinating activity was observed with native cells; however, arcelin did react strongly with some pronase-treated erythrocytes. These results indicate that although arcelin did not have the usual hemagglutinating activity observed for PHA, it did react with receptors that are exposed when some erythrocytes were treated with pronase. PHA showed increased hemagglutinating activity when tested with pronase-treated cells; therefore, the site-specific interaction with these types of cells could be the same for both PHA
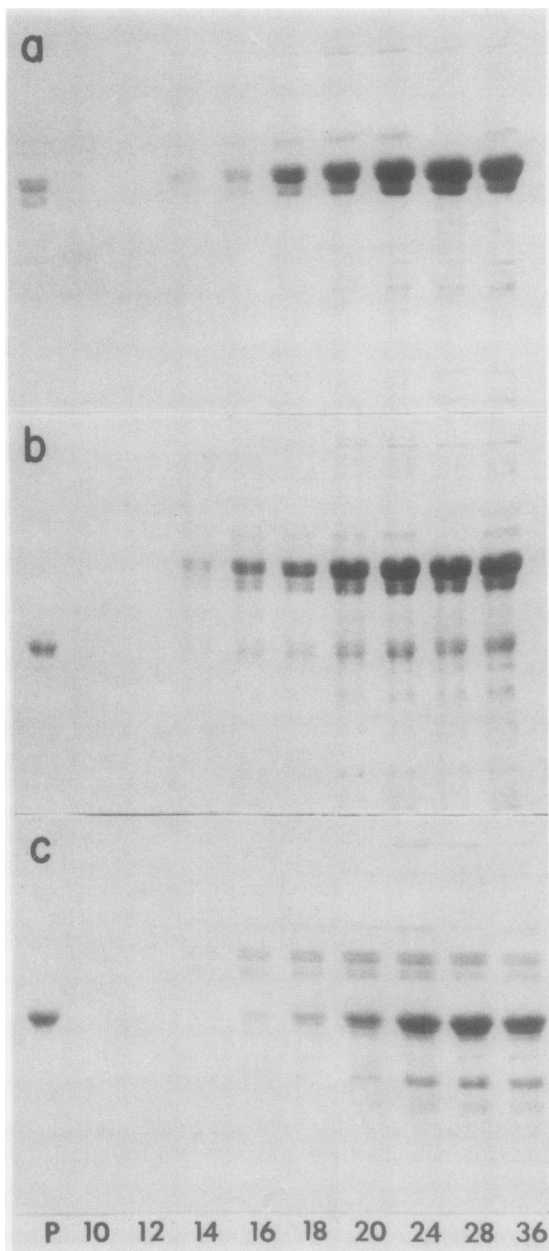

FIG. 6. Separation by SDS-PAGE of crude proteins extracted from developing seeds of L12-56 (panel a), Sanilac (panel b), and SARC1-7 (panel c). Lane P contains purified phaseolin (panel a), PHA (panel b), or arcelin (panel c). Numbers indicate number of days after flowering.

bound arcelin antibody. These proteins were removed during arcelin purification; however, they were observed previously to co-segregate with arcelin protein in F2 progenies (our unpublished data) and may represent low mol wt arcelin proteins. Arcelin antibodies also cross-reacted with purified PHA (Fig. 5b, lane L) and PHA in the crude 'Sanilac' extract (Fig. 5b, lane 2), but did not react with L12-56 crude extract (Fig. 5b, lane 1) or with purified phaseolin (Fig. 5b, lane P).

The observed antibody cross-reaction may have been due to antigenic similarities in carbohydrate groups rather than similarities in protein structure. Therefore, immunoblots were tested using antibodies raised against deglycosylated PHA (Fig. 5c). These antibodies reacted strongly with purified PHA and PHA in the 'Sanilac' and SARC1-7 extracts. There was also cross-reaction with purified arcelin and with one of the low mol wt

and arcelin proteins.

Antigenic similarity between PHA and arcelin proteins was demonstrated by the results of antibody cross-reactions on immunoblots. Antibodies to native arcelin protein cross-reacted with PHA protein and antibodies to deglycosylated PHA cross-reacted with arcelin protein. These results suggest that there are some antigenic similarities in the primary structures of arcelin and PHA proteins.

The accumulation of arcelin protein was compared to phaseolin and PHA accumulation in developing seeds of near isogenic bean lines. The developmental timing of arcelin accumulation in seed of SARC1-7 was similar to that of phaseolin and PHA protein accumulation in seed of L12-56 and 'Sanilac.' Phaseolin accumulation in seed of SARC1-7 terminated earlier than in seed of L12-56 and 'Sanilac.' This may be responsible for the low levels of phaseolin found in seed of arcelin-containing lines (23). The results of this developmental study have been used to isolate messenger RNAs of seed proteins for use in obtaining an arcelin cDNA clone.

The results of this study show that bean arcelin and PHA have several characteristics in common. The two proteins are immunologically related and arcelin has the lectin-like property of agglutinating some sources of pronase-treated erythrocytes. However, arcelin appears to be different than PHA in several respects. One important difference is that arcelin lacks the carbohydrate-binding specificities for native erythrocytes and affinity ligands that are characteristic of PHA. We believe the distinctions between bean arcelin and PHA warrant designation of arcelin as a new and different type of bean seed protein. Nevertheless, these two proteins may share some primary sequence homology that would account for their observed similarities. The relationship between arcelin and PHA will be more fully understood by characterization and comparison of the genes encoding these proteins.

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